

PB84-175306

Histopathology and Enlargement of the
Pituitary of a Teleost Exposed to the
Herbicide Trifluralin

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1984

U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completion)		
1. REPORT NO. EPA-600/J-82-152	2.	3. PB84-175306
4. TITLE AND SUBTITLE HISTOPATHOLOGY AND ENLARGEMENT OF THE PITUITARY OF A TELEOST EXPOSED TO THE HERBICIDE TRIFLURALIN (Journal Version)		5. REPORT DATE 1984
7. AUTHOR(S) J. A. Couch		6. PERFORMING ORGANIZATION CODE
9. PERFORMING ORGANIZATION NAME AND ADDRESS same as 12		8. PERFORMING ORGANIZATION REPORT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory--GB,FL Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, FL 32561		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO.
		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA/600/04
15. SUPPLEMENTARY NOTES REF: Journal of Fish Diseases, 7:157-163, 1984		
16. ABSTRACT Pituitary glands of sheepshead minnows, <i>Cyprinodon variegatus</i> , exposed for 19 months to 1-5 ug/l trifluralin were significantly enlarged and possessed histopathologic characteristics (when compared to glands of controls) such as pseudocysts, congestion of blood vessels and edema. Most of the fish with enlarged pituitaries also had diffuse vertebral hyperostosis and other dysplastic vertebral changes. Several speculative mechanistic paths are suggested for the mode of the effect of trifluralin on the vertebral and pituitary tissues. Study of the form and function of pituitary glands of teleosts from natural populations might provide indications of chronic physiological stress, particularly in relation to chemical pollutant stress.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

Histopathology and enlargement of the pituitary of a teleost exposed to the herbicide trifluralin

J. A. COUCH *United States Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida, U.S.A.*

Abstract. Pituitaries of sheepshead minnows, *Cyprinodon variegatus*, exposed for 19 months to 1-5 µg/l trifluralin in the laboratory exhibited enlargement, pseudocysts, congestion of blood vessels and oedema. Most of the fish with an enlarged pituitary also had induced diffuse and/or focal vertebral hyperostosis and other dysplastic vertebral changes. Several speculative mechanistic paths are suggested for the mode of effect of trifluralin on the vertebral and pituitary tissues. Abnormal enlargement or variation in sizes of pituitaries from fish of the same age, along with histopathological findings may be related to the environmental quality of the fish's habitat, and provide indications of stress within individuals resulting from toxicant exposure.

Introduction

The herbicide trifluralin (2,6 dinitro-N, N-dipropyl-4-(trifluoromethyl) benzamine) has pronounced dysplastic effects on the vertebral column of the sheepshead minnow, *Cyprinodon variegatus* Lacépède, during growth stages (Couch, Winstead, Hansen & Goodman 1979). These dysplastic effects result from exposure to a relatively low concentration for both shorter (days) and longer periods (months) in experimental tests (Couch, Courtney & Foss 1981). The dysplasia may vary, depending upon concentration and duration of exposures, from thickening of vertebral walls and subsequent thickening of whole fish, to focal, asymmetrical hyperossifications along the length of the caudal vertebral region (Couch *et al.* 1981). Similar, confirmatory lesions have been found since in brown trout, *Salmo trutta* L., in nature following trifluralin exposure, and in Atlantic salmon parr, *Salmo salar* L., following laboratory exposure to trifluralin in Scotland (Wells & Cowan 1982).

We have speculated that underlying pathophysiological events stimulated by trifluralin lead to the bony lesions observed in the vertebrae (Couch *et al.* 1979). Without understanding exactly what these events are, but with obvious evidence that the bony lesions are mediated by the persistence and function of osteoblastic cells and possibly notochord tissue, accompanied by elevation of serum calcium (Couch *et al.* 1979), the morphometry and histology of the pituitary from long-term, trifluralin-exposed fish were examined to determine if endocrine gland changes were associated with the vertebral

Correspondence: Dr J. A. Couch, United States Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561, U.S.A.

lesions. The pituitary was chosen because of its central endocrine control, its discrete form and location, and because persistent, direct trifluralin effects (or feedback effects) might be reflected by histology.

Materials and methods

Two groups of *Cyprinodon variegatus* were exposed to trifluralin for 19 months (Table 1), using facilities and methods described by Couch *et al.* (1981). Surviving fish were measured for standard length, examined grossly for external lesions and then fixed directly in Davidson's fixative, after their abdomens were slit open to permit quick penetration of fixative. Brains and attached pituitaries were dissected from preserved fish. Specimens that were damaged during dissection were not used. Each pituitary's length and width was measured from the ventral aspect (Fig. 1). Measurements were made using a dissecting microscope ($\times 25$) and fine needle calipers, calibrated to resolve approximately 1/10 ml. Significance of differences between means of length and width of pituitaries from a single control group and from each of the two experimental groups were determined by *t*-tests with significance criteria set at the 0.01 level. No comparison between the two experimental groups was made. No attempts were made to estimate the volume or weight of each gland because adequate parasagittal or sagittal histological sections required that each pituitary be processed and embedded still attached to the brain. Brains and attached pituitaries were embedded in paraffin wax, sectioned parasagittally and sagittally at 7 μm , and stained with Glenner's stain (Bowling 1967), which differentially stains the various functional, cellular regions of the pituitary.

Results

Vertebral lesion frequencies in groups of fish

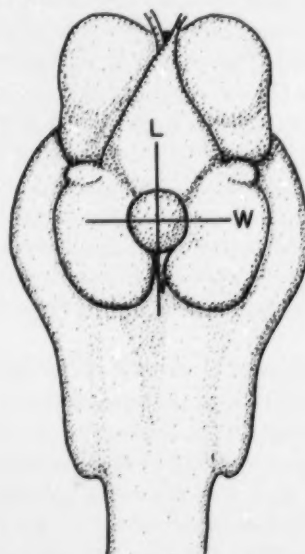
Table 1 lists the numbers of fish in each group with the two major vertebral lesions—diffuse dysplasia and focal hyperostosis. Diffuse dysplasia in the caudal vertebrae of fish in exposure I and exposure II caused grossly observable body thickening in individual fish. Those fish with focal hyperostosis only were diagnosed by radiologic and histologic examination. The frequencies of the two major vertebral lesions in each experimental group (embryo exposure vs adult exposure) were approximately equal (Table 1).

Enlargement of pituitary glands

The mean of the ventral length and width of pituitary glands from each of the exposed groups of fish was significantly greater than the mean length and width of glands from control fish. The size of the pituitary in each fish, however, was not correlated to the standard length or sex of that fish, males and females were approximately equally abundant in each group.

Table 1. Lesions produced in *Cyprinodon variegatus* after 19 months exposure to trifluralin

Group code	Exposed to	Exposure duration	Diffuse vertebral dysplasia	One or more focal vertebral lesions	Combined pituitary enlargement and histopathologic changes
Control	Flowing sea water	Zygote to 19 months	None	None	None
Exposure group I	1-5 mg/l trifluralin in flowing sea water	Zygote to 19 months	17/20	7/20	10/20
Exposure group II	1-5 mg/l trifluralin in flowing sea water	30 days to 19 months	18/20	11/20	11/20

Figure 1. Scheme of ventral view of *Cyprinodon* brain (minus most cranial nerves) to illustrate the aspect from which pituitary measurements were made (lengths and widths).

The quantitative data clearly demonstrate an enlargement of the pituitary related to chronic trifluralin exposure in both experimental groups (Table 2). Parasagittal and sagittal histological sections from control fish pituitary (Fig. 2), when compared to similar sections from trifluralin-exposed fish (Fig. 3), dramatically demonstrate the difference in gland size in similar size fish.

Table 2. Lengths and widths of pituitary glands* from control and exposed *Cyprinodon variegatus* (concentration = 1.5 µg/l trifluralin for 19 months) and mean standard length of fish in each group. Measurements are in mm

Group**	Mean length with ranges	Mean width with ranges	Mean standard length of fish
Control	0.6 (0.3-1.0)	0.6 (0.2-1.0)	40.3
Exposure group I	0.8 (0.5-1.2)	0.9 (0.6-1.2)	42.6
Exposure group II	0.9 (0.5-1.1)	0.9 (0.6-1.9)	40.8

*Using *t*-test, *P* values were less than 0.01 when mean from each exposed group was compared independently to the mean of the control.

***n* = 20 for each group.

Histopathologic characteristics of enlarged pituitaries from exposed fish

The pituitary from control fish was compact and reniform in parasagittal and sagittal sections (Fig. 2). The neurohypophyseal region was moderately vascularized and lightly chromophilic with Glenner's stain. The adenohypophyseal regions of the gland contained a calyx-like region adjacent to the neurohypophysis comprised of dark-blue staining cells, whereas the cortex of the gland contained mainly chromophobic cells (Fig. 2). No demonstrable alpha-like cells were observed in the preparations. Vascular channels in the majority of control fish were small and unremarkable, containing relatively few red blood cells (RBCs). The area occupied by the neurohypophysis in sections of the control fish gland was about equal to the area of adenohypophysis (Fig. 3).

The pituitary from exposed fish was less compact and more irregular in form (Fig. 3). The typical reniform shape of normal glands was lost and the general structural integrity compromised (Fig. 3).

The enlarged pituitary displayed a variety of histopathological changes, with the occurrence of fluid-filled pseudocysts being the most striking. These occurred predominantly in the adenohypophysis (Fig. 3), but also in the neurohypophysis. At least 10 of 20 glands of fish from exposure groups I and II had pseudocysts (1 to 5/pituitary) which were 0.07-0.1 mm in diameter and, in some cases, occupied a significant portion of a glandular region (Figs 3 & 5). Pseudocysts did not contain RBCs and did not appear to be telangiectatic blood vessels (Figs 4 & 5), but often occurred in close association with blood vessels containing RBCs (Fig. 5). Each pseudocyst appeared to possess a limiting membrane (Fig. 5), but not an epithelium. Similar but smaller cysts were seen in three control pituitaries.

Congestion, stasis and dilation of blood vessels were very apparent in pituitaries of exposed fish (Figs 4 & 5). Occasionally, a haemorrhagic area surrounded some of the pseudocysts (Fig. 5). Separation of tissues which were normally compact in the adenohypophysis, possibly caused by fluid pressure, contributed to the loss of normal structure of the gland (Fig. 3).

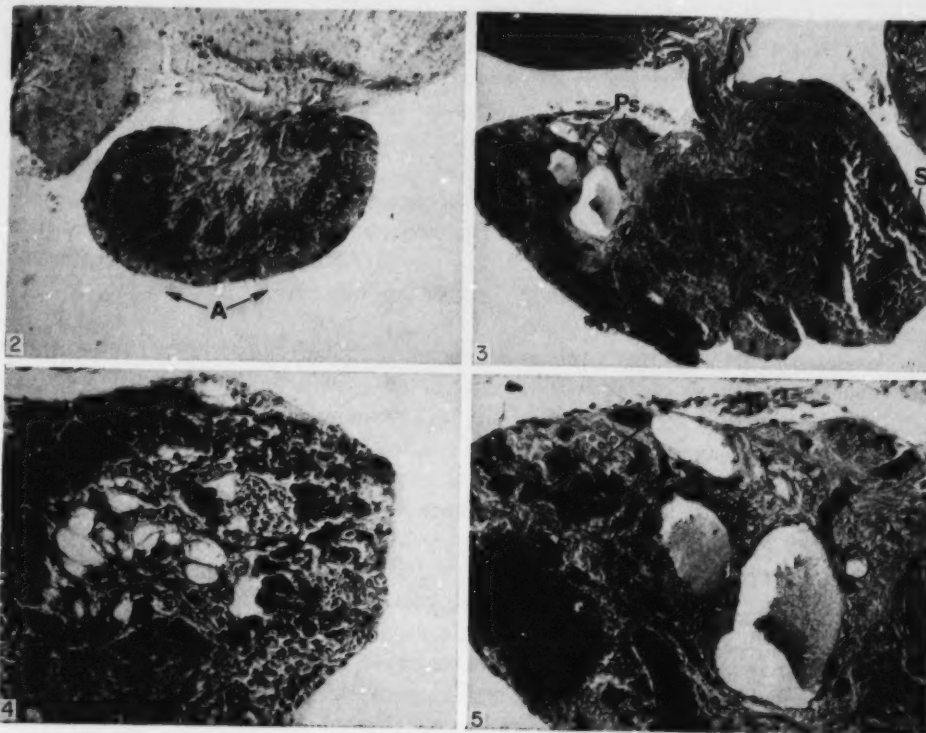


Figure 2. Parasagittal section (almost sagittal) of pituitary gland from control adult fish reared from zygote. Note the compact, reniform shape. Neurohypophysis (N) is approximately equal to adenohypophysis (A) in sectional area (Glenner's, $\times 75$).

Figure 3. Pituitary at comparable location to that in Fig. 2 from fish exposed to trifluralin for 19 months (from zygote). Note the pseudocysts (Ps) and spaces (S) in adenohypophysis (A) (Glenner's, $\times 75$).

Figure 4. Cross-section through anterior lobe of adenohypophysis of exposed fish. Note small pseudocysts (Ps) and associated blood vessels (Glenner's, $\times 186$).

Figure 5. Portion of adenohypophysis of exposed fish. Note stained fluid in pseudocysts and haemorrhagic area around central pseudocyst; also note congested blood vessels and intensely staining foci in outer layer of the adenohypophysis (arrows); note apparent membrane lining pseudocysts (Glenner's, $\times 186$).

Size of lobes, relative to one another, of the adenohypophysis in exposed versus control fish glands did not differ (Figs 2 & 3). These lobes in exposed fish did show abnormal, focal, intense, dark-blue staining with Glenner's stain (Fig. 3).

Discussion

Stolk (1955) reported an increase in size and probably an increase in activity in the

pituitary of swordtail juveniles, *Xiphophorus helleri* Heckel, and guppy adults, *Poecilia reticulata* Peters, exposed to nitrofurazone (static exposure, 69 mg/5000 ml tap water for 45–183 days). Stolk attributed the increase in size of the gland in both fish to both hypertrophy and hyperplasia of the glandular portion (adenohypophysis). I have found no evidence against either hyperplasia or cellular enlargement in the present study. Enlargement of the gland in *Cyprinodon*, however, apparently results from pseudocyst formation, stasis, congestion and oedema, mainly in the adenohypophysis.

The histopathologic changes and enlargement occurred in both fish exposed from zygote to 19 months of age, and in fish exposed from 30 days of age to 19 months. This indicates that the effects described are probably age independent, suggesting a risk for fish exposed to trifluralin at any age.

The pituitary changes in *C. variegatus* occurred concurrently with or subsequent to the development of severe vertebral dysplasia in experimentally exposed fish. Trifluralin may directly affect the osteoblastic and notochordal tissues causing vertebral dysplasia. Another explanation may lie in the fact that each molecule of trifluralin contains three atoms of fluorine which somehow may affect calcium in bone, causing calcium to be displaced (assuming direct uptake of trifluralin by bone in growing fishes, the possibility of which is suggested by known uptake and persistence in whole body of trout as determined by Wells & Cowan [1982]). This may result in calcium metabolic imbalances (release of calcium due to bone rearrangement) that indirectly influence pituitary function and form via endocrine feed-back and compensatory mechanisms; or trifluralin may have a direct effect on other endocrine glands (corpuscle of Stannius, or the ultimobranchial gland in combination with the pituitary) all of which play roles in the mediation and control of calcium metabolism and probably bone formation in growing fish.

Monitoring of both form and function of the pituitary of fish in discrete natural populations might be a useful tool to detect long-term, low-concentration exposures to pollutant chemicals and other stressors.

Acknowledgments

I thank Ms Susan Martin and Mr Lee Courtney for their patient aid in dissecting and measuring the glands.

References

- BOWLING M.C.L. (1967) *Histopathology Laboratory Procedures of the Pathologic Anatomy Branch of the National Cancer Institute*. U.S. Government Printing Office, 0-246-978, Washington, D.C.
- COUCH J.A., COURTNEY L. & FOSS S. (1981) Laboratory evaluation of marine fishes as carcinogen assay subjects. In: *Phyletic Approaches to Cancer* (ed. by C.J. Dawe *et al.*), pp. 125–139. Japan Scientific Society Press, Tokyo.
- COUCH J.A., WINSTEAD J., HANSEN D. & GOODMAN L. (1979) Vertebral dysplasia in young fish exposed to the herbicide trifluralin. *Journal of Fish Diseases* 2, 35–42.

- STOLK A. (1955) Changes in the pituitary gland of the viviparous cyprinodonts *Xiphophorus helleri* Heckel and *Lebistes reticulatus* (Peters) after nitrofurazone treatment. *Proceedings. Koninklijke nederlandse akademie van wetenschappen* **58**, 61-72.
- WELLS D.E. & COWAN A.A. (1982) Vertebral dysplasia in salmonids caused by the herbicide trifluralin. *Environmental Pollution (Series A)* **29**, 249-260.